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Differences in motor unit behavior during isometric contractions in patients with diabetic peripheral neuropathy at various disease severities

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Abstract:

The aim of this study was to determine whether HD-sEMG is sensitive to detecting changes in motor unit behavior amongst healthy adults and type 2 diabetes mellitus (T2DM) patients presenting diabetic peripheral neuropathy (DPN) at different levels. Healthy control subjects (CON, n=8) and T2DM patients presenting no DPN symptoms (ABS, n=8), moderate DPN (MOD, n=18), and severe DPN (SEV, n=12) performed isometric ankle dorsiflexion at 30% maximum voluntary contraction while high-density surface EMG (HD-sEMG) was recorded from the tibialis anterior muscle. HD-sEMG signals were decomposed, providing estimates of discharge rate, motor unit conduction velocity (MUCV), and motor unit territory area (MUTA). As a result, the ABS group presented reduced MUCV compared to CON. The groups with diabetes presented significantly larger MUTA compared to the CON group (p<0.01), and the SEV group presented a significantly lower discharge rate compared to CON and ABS (p<0.01). In addition, the SEV group presented significantly higher CoV_{force} compared to CON and MOD. These results support the use of HD-SEMG as a method to detect peripheral and central changes related to DPN.

Keywords: Diabetic peripheral neuropathy; High-density surface electromyography; conduction velocity; Motor Unit; Motor unit territory area; Discharge rate.

Introduction

Diabetic peripheral neuropathy (DPN) is the most frequent diabetic neuropathy, being defined as a sensory-motor polyneuropathy, symmetric and distal. DPN represents 75% of neuropathy cases, causing sensory and motor deficits (Dyck et al., 2011, 1993; Pop-Busui et al., 2017; Selvarajah et al., 2019; Tesfaye et al., 2010). Motor deficits usually consist of force loss in distal lower limb muscles with a significant proportion of type I fibers (Oberbach et al., 2006), such as the tibialis anterior (TA) (Larsen et al., 2009; Oberbach et al., 2006; Regensteiner et al., 1998). Compromised TA function in DPN affects plantar pressure distribution, ultimately leading to bone deformities. Moreover, the association between bone deformities and dysfunctional plantar pressure distribution may lead to the development of plantar ulcers, considered the primary cause of lower-limb amputations in DPN patients (Fernando et al., 2013; Ferreira et al., 2017; Sacco and Sartor, 2016; Van Schie et al., 2004). Therefore, unraveling deficits in the muscle recruitment of DPN patients can assist in defining better strategies to avoid or at least delay major clinical interventions such as amputations.

Denervation of motor units (MU) is generally linked to force loss in degenerative diseases and aging (Allen et al., 2016; McNeil et al., 2005; Power et al., 2013). However, patients with DPN present a compensatory mechanism involving MU reinnervation to attenuate muscle weakness in the early stages of type II diabetes (Andreassen et al., 2006; Zochodne et al., 2008). The compensatory mechanism increases the MU territory, subsequently increasing muscle fiber density (Bril et al., 1996). Since the motor unit action potential (MUAP) propagation velocity is linearly related to the average diameter of its muscle fibers, the DPN mechanism of denervation and reinnervation may likely

73 influence motor unit conduction velocity (MUCV) (Blijham et al., 2006, 2004; Cruz-
74 Martinez and Arpa, 1999).

75

76 Studies using intramuscular electromyography (iEMG) demonstrated chronic
77 reinnervation in the TA muscle prior to sensory disturbances in type II Diabetes Mellitus
78 (T2DM) patients. It has been shown that chronic reinnervation decreases MFCV while
79 increasing abnormal single-fiber electromyography (*i.e.*, jitter) (Bril et al., 1996; Meijer
80 et al., 2008; Shields, 1987). Moreover, T2DM and DPN patients present abnormalities in
81 their action potentials and motor unit discharge rates in intrinsic muscles of the foot, hand,
82 and the TA (Allen et al., 2013a, 2013b, 2015b). Furthermore, studies using murine models
83 suggested that both MU denervation and sensory dysfunctions occur concomitantly at the
84 initial stages of DPN (Ramji et al., 2007; Souayah et al., 2009). Muscle biopsies are often
85 used to assess muscle properties and provide the extent of muscle degeneration (Gaster
86 et al., 2001; Larsen et al., 2009; Oberbach et al., 2006). However, this technique is
87 invasive, and its results do not inform the status of the muscular neural control properties,
88 such as discharge rate or MFCV. Therefore, it is highly relevant to assess
89 electrophysiological muscular properties of DPN patients non-invasively, as it can
90 potentially become a method to quantify muscle degeneration and its neural control
91 properties.

92

93 The relationship between MFCV and muscle fiber diameter has been previously
94 established through iEMG recordings and muscle biopsies (Blijham et al., 2006).
95 Therefore, EMG recordings can become an alternative method to access relevant
96 information regarding muscle function and its neurophysiology, primarily if non-invasive
97 methods are implemented. Currently, high-density surface electromyography (HD-

sEMG) provides a non-invasive method to identify large MU populations in isometric (Farina et al., 2008, 2001; Merletti et al., 2008; Negro et al., 2016) and dynamic contractions (Oliveira and Negro, 2021). HD-sEMG decomposition allows the estimation of motor unit discharge properties, MUCV with nearly negligible errors (<3%) (Farina et al., 2001), and motor unit territory area (MUTA) (Chandra et al., 2022; Gallina and Vieira, 2015; Kapelner et al., 2016). Therefore, HD-sEMG can be a relevant tool for assessing the properties of muscle recruitment.

The purpose of the present study was to determine whether HD-sEMG is a sensitive method to detect changes in motor unit discharge properties amongst healthy adults and patients at different stages of DPN. We hypothesized that DPN patients would present a reduction in the MUCV and discharge rate and that DPN patients would display an increased MUTA due to the reinnervation mechanisms leading to collateral branching of intact axons. Additionally, we hypothesized that the decrease in MUCV would be more pronounced in subjects with increased DPN severity. The compensatory mechanism that provides reinnervation is ceased at advanced stages of DPN, leading to muscle atrophy due to denervation or disuse (Andersen, 2012; Andersen et al., 1997; Meijer et al., 2008), ultimately reducing MUVC.

Materials and Methods

Participants

Thirty-eight adults with T2DM were recruited from the University Hospital of the Federal University of Santa Catarina. Diabetes patients were divided into four groups with different neuropathy severity: no DPN/absent group (n = 8); moderate DPN group (n = 18) and severe DPN group (n = 12). A simplified version of the NSS (Neuropathy Symptom Score) questionnaire was used to assess the main neuropathic symptoms. Moreover, a modified version of the mNDS test (modified Neuropathy Disability Score) was used to assess the main neuropathic signs. The classification of the severity of DPN was used as absent (symptoms and signs ≤ 2), moderate (3 ≤ symptoms ≤ 7, 3 ≤ signs < 9), and severe (symptoms ≥ 7 and signs ≥ 9) (Abbott et al., 2002; Moreira et al., 2005; Petropoulos et al., 2018; Young et al., 1993). Additionally, eight healthy matching adults (Control group) were recruited from the community.

Anthropometric and clinical data (diabetes mellitus duration, HbA1c test result, use of medications, and presence of complications) were obtained previously from the application of the study protocol. Inclusion criteria for patients: age >40 and < 70 years, T2DM was diagnosed based on the World Health Organization definition (World Health Organization, 2016). Exclusion criteria: minor/major limb amputation or other physical, neurological, musculoskeletal deficiencies, *e.g.*, stroke, cerebral palsy, polio, arthritis, etc.; sight limiting diabetic retinopathy, severe nephropathy, chronic kidney disease stage 4-5, lower limb edema; active diabetic foot ulceration (Butugan et al., 2014). All study procedures followed the principles of the Declaration of Helsinki and were approved by the Human Research Ethics Committee of the Federal University of Santa Catarina

(Protocol Number: 2.390.994). Informed consent was obtained from all participants included in the study.

Experimental Protocol

Participants were instructed to be seated, with the hip and knee flexed at a 90º angle and the ankle in a neutral position at a 90º angle to the leg. The foot of the dominant leg was fixed on a custom dynamometer (Figure 1). After a familiarization session with the equipment and the experimental protocol, participants performed two maximum voluntary isometric contractions (MVIC) for the ankle dorsiflexion with a 5-s duration, interspaced by a 2-minute rest interval. Participants were verbally encouraged to produce the maximum effort throughout the task. The highest force achieved across the two MVICs was defined as the maximum force and used to define submaximal loads. Subsequently, participants performed one 20-s submaximal isometric ankle dorsiflexion at 30% MVIC. Real-time visual force feedback was provided to participants on a computer screen.

"INSERT FIGURE 1 HERE"

Data Acquisition

The HD-sEMG signals were recorded from the TA muscle using a 32-channel EMG system in a monopolar configuration. The sampling frequency was 2000 Hz, 8x gain, and digitized with a 24-bit A/D converter (Favretto et al., 2018). A 13x5 electrode array (ELSCH064NM2, OT Bioelettronica, Torino, Italy, 2 mm diameter, 8 mm inter-electrode distance) was used for the recordings. An 8x4 setup from the array provided the 32-channel EMG signals is shown in Figure 2. Before attaching the matrix, the skin was

cleaned and abraded. The EMG matrix was positioned according to published guidelines (Barbero et al., 2012) and fixed using adhesive tape. The electrodes' cavities formed due to the adhesive were filled with conductive paste (CC1, OT Bioelettronica, Turin, Italy). A reference electrode was fixed on the tuberosity of the tibia. The dynamometer measured the force using a strain-gauge load cell (Model SPL, traction/compression, 60-kg range, sensitivity 2 mV/V, AEPH of Brazil), digitized with a 24-bit A/D converter and a sampling frequency of 80 Hz. The reliability of this dynamometer has been described elsewhere (Andreis et al., 2019).

Data analysis

The acquired signals were analyzed using custom scripts in MATLAB (R2018b, MathWorks, MA, USA). The EMG signals were band-pass filtered (8^{th} order Butterworth, 20-500 Hz) (Merletti, 1999), followed by a notch filter (2^{nd} order, 60 Hz notch filter, and its subsequent five harmonics). The force signals were low pass filtered at 15 Hz using a fourth-order Butterworth filter. The HD-sEMG signals were decomposed into motor unit spike trains using an algorithm based on convolutive blind source separation (Negro et al., 2016). Only motor units with a decomposition accuracy (silhouette) greater than 0.9 were included in the analysis, followed by manual inspection (Boccia et al., 2019; Del Vecchio et al., 2020). The average discharge rate of the interspike interval (ISI) and the ISI coefficient of variation (CoV_{ISI}, ISI standard deviation divided by the ISI average) were calculated (Martinez-Valdes et al., 2015).

The decomposition algorithm identifies the discharge times of each motor unit, but not the waveform of the action potentials. To estimate the action potential waveform of each motor unit, the spike-triggered averaging technique was used. The motor unit action

potential (MUAPs) waveforms were extracted by averaging the monopolar HD-sEMG signals considering rectangular windows of 30 ms, using the discharge times as triggers. The average of the first 20 action potentials of each identified motor unit was used to estimate the motor unit action potential (Farina et al., 2002). From the identified monopolar action potentials, the differential double lead was computed, which is used to calculate the motor unit conduction velocity (MUCV). For the estimation of MUCV, a minimum of 3 and a maximum of 6 differential double leads were used. Double differential derivations are known to provide superior MUCV estimation, as they are less affected by the effects of end fibers and end plates (Farina et al., 2002). The selection criteria for the channels were clear propagation along with the columns of the motor unit action potential and a coefficient of correlation greater than 0.8 (Del Vecchio et al., 2018; Farina et al., 2002). The MUCV was estimated using the multichannel maximum likelihood algorithm, which calculates conduction velocity in MUs with a standard deviation of fewer than 0.1 ms-1 (Farina et al., 2001).

"INSERT FIGURE 2 HERE"

The MUTAs were calculated using the RMS values of each action potential of the decomposed MUs were estimated using a simple differential configuration. The threshold value was selected based on the channel with the highest RMS amplitude within the grid. Then, only values exceeding 50% of the maximum RMS were used. The threshold of 50% value was selected according to the literature (Kapelner et al., 2016). Furthermore, it was observed that a threshold of 50% better demarcated the two-dimensional boundary of the MU territory, which was then segmented with an ellipse. The MUTA was expressed in terms of the number of channels in the electrode matrix (Chandra et al., 2022). In

addition, it was measured the coefficient of variation of the force (CoV_{force}, force standard deviation divided by the mean force) of the submaximal isometric ankle dorsiflexion at 30% MVIC.

Statistical Analysis

Statistical analyses were performed using R (R Code Team, 2018). Data are provided as mean ± standard deviation. The normality of the dependent variables age, body mass index (BMI), MVIC, MVIC/body mass, glycated hemoglobin (HbA1c), DM duration, subcutaneous tissue thickness, MUCV, CoV_{ISI}, MUTA, discharge rate and CoV_{force} were assessed by the Kolmogorov-Smirnov test. Levene's test was used to test for homogeneity of variance across groups. The main effects of groups (Control x absent DPN x moderate DPN x severe DPN) were assessed for all variables (i.e., MUCV, CoV_{ISI}, MUTA, discharge rate, CoV_{force}) using a one-way ANOVA. The Tukey post-hoc test was used if necessary. For categorical data, Fisher's exact test was used. A significance level of 5% was adopted. The *p*-values, *F*-values, and the partial ETA squared (η^2_p) were reported.

Results

There were no significant between-groups differences in gender distribution ($p = 1$), age ($p = 0.26$), BMI ($p = 0.40$) and subcutaneous tissue thickness ($p = 0.64$, Table 1). Moreover, there were no significant between-group differences T2DM duration ($p = 0.84$) and HbA1c ($p = 0.49$), see Table 1.

There were main effects of the group for the mean MVIC (*F*-value = 7, $\eta^2_p = 0.33$, $p < 0.01$, Table 1) and MVIC/body mass (*F*-value = 5.3, $\eta^2_p = 0.28$, $p < 0.01$). The post-hoc analysis revealed that the severe group presented a 52% reduction in MVIC compared to

241 the control group ($p < 0.01$) and a 36% reduction compared to the moderate group ($p <$
242 0.01). Regarding MVIC/body mass, the post-hoc analysis revealed a reduction of
243 approximately 41% for the severe group compared to the control group ($p < 0.01$).
244
245 "INSERT TABLE 1 HERE"
246
247 Illustrative data from MUCV and discharge rates from single individuals in every study
248 group are displayed in Figure 3. The individual with severe DPN presents a reduced
249 MUCV (Figure 3a) as well as reduced discharge rates across the reliably identified motor
250 units compared to participants from the Control, Absent and Moderate groups (Figure
251 3b).
252
253 "INSERT FIGURE 3 HERE"
254
255 No between-group differences were found for CoV_{ISI} ($p = 0.8$). However, there was a
256 main effect of the group for MUCV (F-value = 11.8, $\eta^2_p = 0.46$, $p < 0.01$, Figure 4(a)).
257 The post-hoc analysis revealed that the MUCV from the severe group was lower
258 compared to the control (average difference: -18%) and moderate group (-13%).
259 Moreover, the absent group presented a 10% reduction in the MUCV compared to the
260 control group. Regarding the MUTA, there was a main effect of the group (F-value = 4.2,
261 $\eta^2_p = 0.23$, $p = 0.01$) (Figure 4(b)). Following the post-hoc analysis, the MUTA was larger
262 for the absent (27 %), moderate (27 %), and severe groups (28 %) compared to the control
263 group.
264
265 "INSERT FIGURE 4 HERE"
266

There was a main effect of the group for the mean discharge rate (F-value = 4.4, η^2_p = 0.24, $p < 0.01$) (Figure 5(a)). The post-hoc analysis exhibited that the discharge rate from the severe group was lower when compared to the absent (-19%) and the control groups (-18%). In addition, there was a main effect of the group for the CoV_{force} (F-value = 6.3, $\eta^2_p = 0.31$, $p < 0.01$) (Figure 5(b)). According to the post-hoc analysis, the CoV_{force} from the severe group was larger compared to the control (67%) and the moderate groups (37%).

"INSERT FIGURE 5 HERE"

Discussion

The purpose of this study was to determine whether HD-sEMG can be a suitable method to detect changes in motor unit recruitment properties in T2DM patients with DPN at different disease severities. The main findings of this study were that the group with absent DPN signs presented reduced MUCV and increased MUTA while maintaining discharge rate levels similar to controls. In addition, the moderate DPN group presented similar MUCV, discharge rate and CoV_{force} when compared to the controls, but exhibited greater MUTA. Furthermore, patients with severe DPN presented reduced MUCV and discharge rate, along with increased MUTA and CoV_{force} force compared to the control group. These results suggest that DPN may initially compromise only peripheral/morphological (i.e., motor unit conduction velocity) properties of muscle recruitment, leading to combined peripheral and central (i.e., motor unit discharge rate) deficits only at the later stage of the disease. Moreover, our study demonstrates that the decomposition of HD-sEMG signals is a relevant method for detecting changes in motor unit recruitment properties in T2DM patients presenting different DPN stages.

292 The reductions in force with the DPN progression in our study corroborate previous
293 investigations (Andersen et al., 2004; Andreassen et al., 2006). Interestingly, it is possible
294 to associate the changes in maximal force with muscle recruitment properties. Firstly,
295 there is a reduction in force already in the absent DPN group, which is accompanied by a
296 reduction in MUCV. MUCV reductions in T2DM patients presenting no signs of DPN
297 may be explained by ongoing axonal losses and subsequent muscle fiber atrophy due to
298 denervation and disuse (Andersen, 2012; Andersen et al., 1997; Meijer et al., 2008). From
299 absent to moderate DPN, there is the maintenance of force levels, and the moderate group
300 demonstrated similar MUCV. However, an increased MUTA was observed. Previous
301 studies have described a compensatory reinnervation mechanism in DPN patients, in
302 which denervated muscle fibers originally from low-threshold motor units are
303 reinnervated by high-threshold motor units, resulting in an increased MUTA (Allen et al.,
304 2015; Oberbach et al., 2006; Gaster et al., 2001 Andersen et al., 2009). This mechanism
305 contributes to the maintenance of force levels and increases in the MUCV. Finally, the
306 severe group generates even lower MVIC as a result of generalized muscle atrophy and
307 neuronal losses (Meijer et al., 2008). Although the severe group also shows an increase
308 in the MUTA, the lower MVC values may be related to the failure of the compensatory
309 reinnervation mechanism and, consequently, generalized muscle atrophy (Allen et al.,
310 2014; Andersen, 2012; Andersen et al., 1997; Meijer et al., 2008), which were illustrated
311 in the present study by reduced motor unit discharge rate and motor unit conduction
312 velocity.
313
314 Contrary to our initial hypothesis, MUCV did not gradually decrease as a function of
315 DPN progression towards the severe stages. Instead, there were only specific significant
316 reductions for the absent and the severe DPN groups. Interestingly, a reduction in MFCV

317 in the absent group has been previously reported (Butugan et al., 2014; Meijer et al., 2008;
318 Suda et al., 2016). As previously mentioned, MUCV reductions in T2DM patients are
319 related to axonal losses, muscle fiber denervation and disuse (Allen et al., 2014;
320 Andersen, 2012; Andersen et al., 1997; Meijer et al., 2008), which underlines a peripheral
321 adaptation in such patients. Moreover, T2DM is known to affect the composition of slow-
322 twitch type I fibers (Gaster et al., 2001; Larsen et al., 2009; Oberbach et al., 2006). This
323 compromise in muscle composition should be amplified in our study, as the TA muscle
324 presents a greater proportion of type I fibers (Johnson et al., 1973). Therefore, the
325 reduction in MUCV in the absence of DPN signs may be attributed to T2DM-related
326 peripheral changes in muscle fiber content.
327
328 Interestingly, the MUCV from patients in the moderate group was generally the highest
329 and statistically similar to the MUCV from patients in the control group (see Figure 4(a)).
330 Indeed, Suda et al. (2016) reported an increase in MFCV in individuals presenting severe
331 stages of DPN, which may be related to muscle fiber regeneration. Furthermore, it has
332 been shown that T2DM may cause reductions in the proportion of lower diameter fibers
333 with a corresponding increase in the proportion of higher diameter fibers as part of a
334 compensatory reinnervation mechanism (Gaster et al., 2001; Larsen et al., 2009;
335 Oberbach et al., 2006). Therefore, the increased MFCV in patients at the moderate DPN
336 severity level may be related to the denervation of low-threshold motor units and the
337 reinnervation of the available type of slow-twitch fibers (Suda et al., 2016) by high-
338 threshold motor units. This result is highly relevant, as individuals with moderate DPN
339 may be able to adapt to DPN-related deficits in motor performance through naturally
340 occurring peripheral compensatory mechanisms. Future studies testing the effects of
341 resistance/strength training on motor unit discharge properties in moderate DPN patients

342 can substantially contribute to minimizing motor deficits in DPN patients, potentially
343 avoiding its progression towards severe DPN.
344
345 The motor unit discharge rate represents the descending command from the central
346 nervous system to elicit muscle contractions (Heckman and Enoka, 2012), therefore
347 considered a central component for motor unit function and control. As expected, the
348 severe DPN group generated lower discharge rates during stable isometric contractions
349 than the control and absent groups. This result corroborates previous studies investigating
350 low-force isometric contractions for the TA (Allen et al., 2013b, 2014, 2015b) and vastus
351 lateralis muscle (Watanabe et al., 2013) of patients with DPN. The decreased discharge
352 rate in DPN patients has been attributed to changes in the contractile properties of muscle
353 fibers (Allen et al., 2014; Watanabe et al., 2013), as well as to changes in Na+/K+ pump
354 function resulting in conduction failure in patients with diabetic neuropathy (Krishnan et
355 al., 2008). In addition, the results from our study demonstrated an increased MUTA in
356 the TA muscle at all severity levels. This result suggests that a given force output may be
357 reached by a smaller number of motor units recruited at lower firing rates (Allen et al.,
358 2013b). The loss of motor units and the increase in MUTA may also explain the increase
359 in force variability (CoV_{force}) (Figure 5(b)) for the severe group in relation to the control
360 group (Figure 5(b)), as a smaller number of MUs may result in a less effective averaging
361 process (Negro et al., 2009) and therefore poorer force control. This result corroborates
362 the findings of previous research that reported greater variability at low levels of isometric
363 dorsiflexion contractions in diabetic subjects with severe DPN (Suda et al., 2017) and

higher torque variability in individuals with type 2 DM without DPN (Senefeld et al., 2020).

Regarding asymptomatic DPN, increased variability in motor unit discharge rate has been found in patients with T2DM but asymptomatic to DPN, suggesting early neurophysiological DPN symptoms (Senefeld et al., 2020). However, our results regarding variability in inter-spike intervals did not reveal similar results. Nonetheless, our study demonstrated that non-invasive HD-sEMG was sensitive to underpin differences between healthy or patients with T2DM without DPN and those with considerable DPN progression, highlighting the potential of this technique to contribute to clinical research and practice.

The expected worsened performance for the severe DPN group has been confirmed by the reduced MVIC, MUCV, and discharge rate. In the severe stage of DPN, the denervation process is more effective than the previously mentioned reinnervation mechanism in place for moderate DPN, causing muscle atrophy and weakness due to the death of orphaned muscle fibers (Allen et al., 2016). Furthermore, previous studies have reported the loss of power, contractile quality, and muscle endurance in patients with DPN (Allen et al., 2015a, 2014; Hilton et al., 2008; Moore et al., 2016). In addition, there have been some suggestions on the association between T2DM and motor neuron dysfunctions that can ultimately lead to amyotrophic lateral sclerosis (Lekoubou et al., 2014; Logroscino, 2015; Sun et al., 2015). Motor neuron disorders may also influence

motor unit recruitment and compromise ideal motor unit firing during isometric contractions, partially explaining reductions in motor unit discharge rate.

A limitation of the study is the lack of HD-sEMG measurements of the ramp contractions performed to reach and leave the target force level (30% MVIC). Evaluating the ramp phases of the contraction would allow to investigate disease-related changes in rate coding and motor unit common input/synchronization across the T2DM severity groups. Future studies evaluating the rate coding at these different groups are necessary to elucidate the issue.

Conclusion

We conclude that individuals with DPN presented changes in the MUCV, MUTA, CoV_{force} and discharge rate. The T2DM patients with no signs of DPN already present neurophysiological signs of the disease presence, demonstrated by the reduced MUCV and increased MUTA for this patient group. Moreover, the moderate DPN group presented a similar MUCV and increased MUTA compared to patients in the control group, corroborating that reinnervation mechanisms may play a crucial role in maintaining motor function at this stage of the disease. Finally, the generalized detriment of motor unit recruitment properties in patients with severe DPN has been confirmed by the reduced discharge rate and MUCV, and increased MUTA and CoV_{force}. Therefore, we found evidence to support the use of HD-SEMG as a method to detect DPN-related changes in motor unit recruitment properties. However, more studies are needed to

improve our understanding of the neurophysiological adaptations caused by the increasing severity of DPN.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abbott, C.A., Carrington, A.L., Ashe, H., Bath, S., Every, L.C., Griffiths, J., Hann, A.W., Hussein, A., Jackson, N., Johnson, K.E., Ryder, C.H., Torkington, R., Van Ross, E.R.E., Whalley, A.M., Widdows, P., Williamson, S., Boulton, A.J.M., 2002. The North-West Diabetes Foot Care Study: Incidence of, and risk factors for, new diabetic foot ulceration in a community-based patient cohort. Diabet. Med. 19, 377–384. https://doi.org/10.1046/j.1464-5491.2002.00698.x

Allen, M.D., Choi, I.H., Kimpinski, K., Doherty, T.J., Rice, C.L., 2013a. Motor unit loss and weakness in association with diabetic neuropathy in humans. Muscle Nerve 48, 298–300. https://doi.org/10.1002/mus.23792

Allen, M.D., Doherty, T.J., Rice, C.L., Kimpinski, K., 2016. Physiology in Medicine: neuromuscular consequences of diabetic neuropathy. J. Appl. Physiol. 121, 1–6. https://doi.org/10.1152/japplphysiol.00733.2015

Allen, M.D., Kimpinski, K., Doherty, T.J., Rice, C.L., 2015a. Decreased muscle endurance associated with diabetic neuropathy may be attributed partially to neuromuscular transmission failure. J. Appl. Physiol. 118, 1014–1022. https://doi.org/10.1152/japplphysiol.00441.2014

Allen, M.D., Kimpinski, K., Doherty, T.J., Rice, C.L., 2013b. Length dependent loss of motor axons and altered motor unit properties in human diabetic polyneuropathy. Clin. Neurophysiol. 125, 836–843. https://doi.org/10.1016/j.clinph.2013.09.037

Allen, M.D., Major, Brendan, Kimpinski, Kurt, Doherty, T.J., Rice, C.L., Major, B, Kimpinski, K, Tj, D., Skeletal, R.C.L., 2014. Skeletal muscle morphology and contractile function in relation to muscle denervation in diabetic neuropathy. J Appl Physiol 116 116, 545–552. https://doi.org/10.1152/japplphysiol.01139.2013

Allen, M.D., Stashuk, D.W., Kimpinski, K., Doherty, T.J., Hourigan, M.L., Rice, C.L., 2015b. Increased neuromuscular transmission instability and motor unit remodelling with diabetic neuropathy as assessed using novel near fibre motor unit potential parameters. Clin. Neurophysiol. 126, 794–802. https://doi.org/10.1016/j.clinph.2014.07.018

Andersen, H., 2012. Motor dysfunction in diabetes. Diabetes Metab. 28, 89–92. https://doi.org/10.1002/dmrr

Andersen, H., Gadeberg, P.C., Brock, B., Jakobsen, J., 1997. Muscular atrophy in diabetic neuropathy: a stereological magnetic resonance imaging study. Diabetologia 40, 1062–1069. https://doi.org/10.1007/s001250050788

Andersen, H., Nielsen, S., Mogensen, C.E., Jakobsen, J., 2004. Muscle Strength in Type 2 Diabetes. Diabetes 53, 1543–1548. https://doi.org/10.2337/diabetes.53.6.1543

Andreassen, C.S., Jakobsen, J., Andersen, H., 2006. Muscle Weakness: A Progressive Late Complication in Diabetic Distal Symmetric Polyneuropathy. Diabetes 55, 806–812. https://doi.org/10.2337/diabetes.55.03.06.db05-1237

Andreis, F.R., Favretto, M.A., Cossul, S., Barbetta, P.A., Marques, J.L.B., 2019. Reliability of Maximal Voluntary Isometric Contraction of Ankle Dorsiflexion in Male Subjects. IFMBE Proc. 333–349. https://doi.org/10.1007/978-3-319-77335-3

464 Barbero, M., Merletti, R., Rainoldi, A., 2012. Atlas of Muscle Innervation Zones 1–146.
465 https://doi.org/10.1017/CBO9781107415324.004

466 Blijham, P.J., Hengstman, G.J.D., Laak, H.J.T.E.R., 2004. Muscle-fiber Conduction
467 Velocity and Electromyography as Diagnostic Tools in Patients with Suspected
468 Inflammatory Myopathy: A Prospective Study. Muscle Nerve 29, 46–50.

469 Blijham, P.J., Laak, H.J., Schelhaas, H.J., Engelen, B.G.M. Van, Stegeman, D.F., Zwarts,
470 M.J., Stegeman, D.F., Relation, M.J.Z., 2006. Relation between muscle fiber
471 conduction velocity and fiber size in neuromuscular disorders. Am. Physiol. Soc.
472 100, 1837–1841. https://doi.org/10.1152/japplphysiol.01009.2005.

473 Boccia, G., Martinez-Valdes, E., Negro, F., Rainoldi, A., Falla, D., 2019. Motor unit
474 discharge rate and the estimated synaptic input to the vasti muscles is higher in open
475 compared with closed kinetic chain exercise. J. Appl. Physiol. 127, 950–958.
476 https://doi.org/10.1152/japplphysiol.00310.2019

477 Bril, V., Werb, M.R., Greene, D.A., Sima, A.A.F., 1996. Single-fiber electromyography
478 in diabetic peripheral polyneuropathy. Muscle Nerve 19, 2–9.
479 https://doi.org/10.1002/(SICI)1097-4598(199601)19:1<2::AID-MUS1>3.0.CO;2-J

480 Butugan, M.K., Sartor, C.D., Watari, R., Cecília, M., Martins, S., Ortega, N.R.S.,
481 Vigneron, V.A.M., Sacco, I.C.N., 2014. Multichannel EMG-based estimation of
482 fiber conduction velocity during isometric contraction of patients with different
483 stages of diabetic neuropathy. J. Electromyogr. Kinesiol. 24, 465–472.
484 https://doi.org/10.1016/j.jelekin.2014.04.007

485 Chandra, S., Suresh, N.L., Afsharipour, B., Rymer, W.Z., Holobar, A., 2022. Anomalies
486 of motor unit amplitude and territory after botulinum toxin injection. J. Neural Eng.
487 1–11.

488 Cruz-Martinez, A., Arpa, J., 1999. Muscle fiber conduction velocity in situ (MFCV) in
489 denervation, reinnervation and disuse atrophy. Acta Neurol. Scand. 100, 337–340.

490 Daube, J.R., Rubin, D.I., 2009. Needle electromyography. Muscle and Nerve 39, 244–
491 270. https://doi.org/10.1002/mus.21180

492 Del Vecchio, A., Holobar, A., Falla, D., Felici, F., Enoka, R.M., Farina, D., 2020.
493 Tutorial: Analysis of motor unit discharge characteristics from high-density surface
494 EMG signals. J. Electromyogr. Kinesiol. 53, 102426.
495 https://doi.org/10.1016/j.jelekin.2020.102426

496 Del Vecchio, A., Negro, F., Felici, F., Farina, D.., 2018. Distribution of muscle fibre
497 conduction velocity for representative samples of motor units in the full recruitment
498 range of the tibialis anterior muscle. Acta Physiol. Scand. 222, 1–9.
499 https://doi.org/10.1111/apha.12930

500 Dyck, P., Albers, J.W., Andersen, H., Arezzo, J.C., Biessels, G.-J., Bril, V., Feldman,
501 E.L., Litchy, W.J., O'Brien, P.C., Russell, J.W., 2011. Diabetic polyneuropathies:
502 update on research definition, diagnostic criteria and estimation of severity Peter.
503 Diabetes. Metab. Res. Rev. 26, 620–628. https://doi.org/10.1002/dmrr

504 Dyck, P.J., Kratz, K.M., Karnes, J.L., Litchy, W.J., Klein, R., Pach, J.M., Wilson, D.M.,
505 O'Brien, P.C., Melton, L.J., Service, F.J., 1993. The prevalence by staged severity
506 of various types of diabetic neuropathy, retinopathy, and nephropathy in a

population-based cohort: the Rochester Diabetic Neuropathy Study. Neurology 43, 817–24.

Farina, D., Arendt-Nielsen, L., Merletti, R., Graven-Nielsen, T., 2002. Assessment of single motor unit conduction velocity during sustained contractions of the tibialis anterior muscle with advanced spike triggered averaging. J. Neurosci. Methods 115, 1–12. https://doi.org/10.1016/S0165-0270(01)00510-6

Farina, D., Leclerc, F., Arendt-Nielsen, L., Buttelli, O., Madeleine, P., 2008. The change in spatial distribution of upper trapezius muscle activity is correlated to contraction duration. J. Electromyogr. Kinesiol. 18, 16–25. https://doi.org/10.1016/j.jelekin.2006.08.005

Farina, D., Muhammad, W., Fortunato, E., Mestre, O., Merletti, R., Rix, H., 2001. Estimation of single motor unit conduction velocity from surface electromyogram signals detected with linear electrode arrays. Med. Biol. Eng. Comput. 39, 225–236.

Favretto, M.A., Cossul, S., Andreis, F.R., Balotin, A.F., Marques, J.L.B., 2018. High Density Surface EMG System Based on ADS1298-front end. IEEE Lat. Am. Trans. 16, 1616–1622. https://doi.org/10.1109/TLA.2018.8444157

Fernando, M., Crowther, R., Lazzarini, P., Sangla, K., Cunningham, M., Buttner, P., Golledge, J., 2013. Biomechanical characteristics of peripheral diabetic neuropathy : A systematic review and meta-analysis of findings from the gait cycle , muscle activity and dynamic barefoot plantar pressure. Clin. Biomech. 28, 831–845. https://doi.org/10.1016/j.clinbiomech.2013.08.004

Ferreira, J.P., Sartor, C.D., Leal, Â.M.O., Sacco, I.C.N., Sato, T.O., Ribeiro, I.L., Soares, A.S., Cunha, J.E., Salvini, T.F., 2017. The effect of peripheral neuropathy on lower limb muscle strength in diabetic individuals. Clin. Biomech. 43, 67–73. https://doi.org/10.1016/j.clinbiomech.2017.02.003

Gallina, A., Vieira, T., 2015. Territory and fiber orientation of vastus medialis motor units: A Surface electromyography investigation. Muscle and Nerve 52, 1057–1065. https://doi.org/10.1002/mus.24662

Gaster, M., Staehr, P., Beck-nielsen, H., Schrøder, H.D., Handberg, A., 2001. GLUT4 Is Reduced in Slow Muscle Fibers of Type 2 Diabetic Patients 1324–1329.

Heckman, C.J., Enoka, R.M., 2012. Motor unit. Compr. Physiol. 2, 2629–2682. https://doi.org/10.1002/cphy.c100087

Helliwell, T.R., Coakley, J., Smith, P.E.M., Edwards, R.H.T., 1987. The morphology and morphometry of the normal human tibialis anterior muscle. Neuropathol. Appl. Neurobiol. 13, 297–307. https://doi.org/10.1111/j.1365-2990.1987.tb00069.x

Hilton, T.N., Tuttle, L.J., Bohnert, K.L., Mueller, M.J., Sinacore, D.R., 2008. Excessive Adipose Tissue Infiltration in Skeletal Muscle in Individuals With Obesity, Diabetes Mellitus, and Peripheral Neuropathy: Association With Performance and Function. Phys. Ther. 88, 1336–1344. https://doi.org/10.2522/ptj.20080079

Jaworowski, Å., Porter, M.M., Holmbäck, A.M., Downham, D., Lexell, J., 2002. Enzyme activities in the tibialis anterior muscle of young moderately active men and women: Relationship with body composition, muscle cross-sectional area and fibre type composition. Acta Physiol. Scand. 176, 215–225. https://doi.org/10.1046/j.1365-

201X.2002.t01-2-01004.x

Johnson, M.A., Polgar, J., Weightman, D., Appleton, D., 1973. Data on the distribution of fibre types in thirty-six human muscles. An autopsy study. J. Neurol. Sci. 18, 111–129. https://doi.org/10.1016/0022-510X(73)90023-3

Kapelner, T., Jiang, N., Holobar, A., Vujaklija, I., Roche, A.D., Farina, D., Aszmann, O.C., 2016. Motor unit characteristics after targeted muscle reinnervation. PLoS One 11, 1–12. https://doi.org/10.1371/journal.pone.0149772

Krishnan, A. V, Cindy, S., Kiernan, M.C., 2008. Activity-dependent excitability changes suggest Na + / K + pump dysfunction in diabetic neuropathy. Brain Res. 131, 1209–1216. https://doi.org/10.1093/brain/awn052

Larsen, S., Ara, I., Rabøl, R., Andersen, J.L., Boushel, R., Dela, F., Helge, J.W., 2009. Are substrate use during exercise and mitochondrial respiratory capacity decreased in arm and leg muscle in type 2 diabetes? Diabetologia 52, 1400–1408. https://doi.org/10.1007/s00125-009-1353-4

Lekoubou, A., Matsha, T.E., Sobngwi, E., Kengne, A.P., 2014. Effects of diabetes mellitus on amyotrophic lateral sclerosis: A systematic review. BMC Res. Notes 7, 2–9. https://doi.org/10.1186/1756-0500-7-171

Logroscino, G., 2015. Motor neuron disease: Are diabetes and amyotrophic lateral sclerosis related? Nat. Rev. Neurol. 11, 488–489. https://doi.org/10.1038/nrneurol.2015.145

Martinez-Valdes, E., Laine, C.M., Falla, D., Mayer, F., Farina, D., 2015. High-density surface electromyography provides reliable estimates of motor unit behavior. Clin. Neurophysiol. 127, 2534–2541. https://doi.org/10.1016/j.clinph.2015.10.065

McNeil, C.J., Doherty, T.J., Stashuk, D.W., Rice, C.L., 2005. Motor unit number estimates in the tibialis anterior muscle of young, old, and very old men. Muscle and Nerve 31, 461–467. https://doi.org/10.1002/mus.20276

Meijer, J.W.G., Lange, F., Links, T.P., Hoeven, J.H. Van Der, 2008. Muscle fiber conduction abnormalities in early diabetic polyneuropathy. Clin. Neurophysiol. 119, 1379–1384. https://doi.org/10.1016/j.clinph.2008.02.003

Merletti, R., 1999. Standards for Reporting EMG Data. J. Electromyogr. Kinesiol. 9. https://doi.org/10.1016/S1050-6411(17)30329-2

Merletti, R., Holobar, A., Farina, D., 2008. Analysis of motor units with high-density surface electromyography. J. Electromyogr. Kinesiol. 18, 879–890. https://doi.org/10.1016/j.jelekin.2008.09.002

Moore, C.W., Allen, M.D., Kimpinski, K., Doherty, T.J., Rice, C.L., 2016. Reduced skeletal muscle quantity and quality in patients with diabetic polyneuropathy assessed by magnetic resonance imaging. Muscle and Nerve 53, 726–732. https://doi.org/10.1002/mus.24779

Moreira, R.O., Castro, A.P., Papelbaum, M., Appolinário, J.C., Ellinger, V.C.M., Coutinho, W.F., Zagury, L., 2005. Tradução para o Português e Avaliação da Confiabilidade de Uma Escala para Diagnóstico da Polineuropatia Distal Diabética. Arq Bras Endocrinol Metab vol 49, 944–950. https://doi.org/2005;49/6:944-950

Nandedkar, S.D., Stalberg, E., 1983. Simulation of single muscle fibre action potentials. Med. Biol. Eng. Comput. 21, 158–165. https://doi.org/10.1007/BF02441531

Negro, F., Holobar, A., Farina, D., 2009. Fluctuations in isometric muscle force can be described by one linear projection of low-frequency components of motor unit discharge rates. J. Physiol. 587, 5925–5938. https://doi.org/10.1113/jphysiol.2009.178509

Negro, F., Muceli, S., Castronovo, A.M., Holobar, A., Farina, D., 2016. Multi-channel intramuscular and surface EMG decomposition by convolutive blind source separation. J. Neural Eng. 13. https://doi.org/10.1088/1741-2560/13/2/026027

Oberbach, A., Bossenz, Y., Lehmann, S., Niebauer, J., Adams, V., Paschke, R., Schön, M.R., Blüher, M., Punkt, K., 2006. Altered Fiber Distribution and Fiber-Specific Glycolytic and Oxidative Enzyme Activity in Skeletal Muscle of Patients With Type 2 Diabetes. Diabetes Care 29, 895–900. https://doi.org/10.2337/diacare.29.04.06.dc05-1854

Oliveira, A.S., Negro, F., 2021. Neural control of matched motor units during muscle shortening and lengthening at increasing velocities. J. Appl. Physiol. 130, 1798–1813. https://doi.org/10.1152/japplphysiol.00043.2021

Petropoulos, I.N., Ponirakis, G., Khan, A., Almuhannadi, H., Gad, H., Malik, R.A., 2018. Diagnosing diabetic neuropathy: Something old, something new. Diabetes Metab. J. 42, 255–269. https://doi.org/10.4093/dmj.2018.0056

Pop-Busui, R., Boulton, A.J.M., Feldman, E.L., Bril, V., Freeman, R., Malik, R.A., Sosenko, J.M., Ziegler, D., 2017. Diabetic neuropathy: A position statement by the American diabetes association. Diabetes Care 40, 136–154. https://doi.org/10.2337/dc16-2042

Power, G.A., Dalton, B.H., Rice, C.L., 2013. Human neuromuscular structure and function in old age : A brief review. J Sport Heal. Sci 2, 215–226. https://doi.org/10.1016/j.jshs.2013.07.001.Human

R Code Team, 2018. A Language and Environment for Statistical Computing.

Ramji, N., Toth, C., Kennedy, J., Zochodne, D.W., 2007. Does diabetes mellitus target motor neurons ? Neurobiol. Dis. 26, 301–311. https://doi.org/10.1016/j.nbd.2006.11.016

Regensteiner, J.G., Bauer, T. a, Reusch, J.E., Brandenburg, S.L., Sippel, J.M., Vogelsong, a M., Smith, S., Wolfel, E.E., Eckel, R.H., Hiatt, W.R., 1998. Abnormal oxygen uptake kinetic responses in women with type II diabetes mellitus. J. Appl. Physiol. 310–317. https://doi.org/10.1152/jappl.1998.85.1.310

Sacco, I.C.N., Sartor, C.D., 2016. From treatment to preventive actions: improving function in patients with diabetic polyneuropathy. Diabetes. Metab. Res. Rev. 32, 13–23. https://doi.org/10.1002/dmrr

Selvarajah, D., Kar, D., Khunti, K., Davies, M.J., Scott, A.R., Walker, J., Tesfaye, S., 2019. Diabetic peripheral neuropathy: advances in diagnosis and strategies for screening and early intervention. LANCET Diabetes Endocrinol. 8587. https://doi.org/10.1016/S2213-8587(19)30081-6

634 Senefeld, J.W., Keenan, K.G., Ryan, K.S., D'Astice, S.E., Negro, F., Hunter, S.K., 2020.
635 Greater fatigability and motor unit discharge variability in human type 2 diabetes.
636 Physiol. Rep. 8, 1–15. https://doi.org/10.14814/phy2.14503

637 Shields, R.W., 1987. Single-fiber electromyography is a sensitive indicator of axonal
638 degeneration in diabetes. Neurology 37, 1394–1394.
639 https://doi.org/10.1212/WNL.37.8.1394

640 Souayah, N., Potian, J.G., Garcia, C.C., Krivitskaya, N., Boone, C., Routh, V.H.,
641 Mcardle, J.J., 2009. Motor unit number estimate as a predictor of motor dysfunction
642 in an animal model of type 1 diabetes. Am. J. Physiol. 297, 602–608.
643 https://doi.org/10.1152/ajpendo.00245.2009.

644 Suda, E.Y., Gomes, A.A., Butugan, M.K., Sacco, I.C.N., 2016. Muscle fiber conduction
645 velocity in different gait phases of early and late-stage diabetic neuropathy. J.
646 Electromyogr. Kinesiol. 30, 263–271. https://doi.org/10.1016/j.jelekin.2016.08.006

647 Sun, Y., Lu, C.J., Chen, R.C., Hou, W.H., Li, C.Y., 2015. Risk of amyotrophic lateral
648 sclerosis in patients with diabetes: A nationwide population-based cohort study. J.
649 Epidemiol. 25, 445–451. https://doi.org/10.2188/jea.JE20140176

650 Tesfaye, S., Boulton, A.J.M., Dyck, P.J., Freeman, R., Horowitz, M., Kempler, P., Lauria,
651 G., Malik, R.A., Spallone, V., Vinik, A., Bernardi, L., Valensi, P., 2010. Diabetic
652 neuropathies: Update on definitions, diagnostic criteria, estimation of severity, and
653 treatments. Diabetes Care 33, 2285–2293. https://doi.org/10.2337/dc10-1303

654 Van Schie, C.H.M., Vermigli, C., Carrington, A., Boulton, A., 2004. Muscle Weakness
655 and Foot Deformities in Diabetes. Diabetes Care 27, 1668–73.
656 https://doi.org/10.2337/diacare.27.7.1668

657 Watanabe, K., Gazzoni, M., Holobar, A., Miyamoto, T., Fukuda, K., Merletti, R.,
658 Moritani, T., 2013. Motor unit firing pattern of vastus lateralis muscle in type 2
659 diabetes mellitus patients. Muscle Nerve 48, 806–813.
660 https://doi.org/10.1002/mus.23828

661 World Health Organization, 2016. Global Report on Diabetes. World Heal. Organ. 978,
662 88. https://doi.org/ISBN 978 92 4 156525 7

663 Young, M.J., Boulton, A.J.M., Macleod, A.F., Williams, D.R.R., Onksen, P.H.S., 1993.
664 A multicentre study of the prevalence of diabetic peripheral neuropathy in the United
665 Kingdom hospital clinic population. Diabetologia 36, 150–154.

666 Zochodne, D.W., Ramji, N., Toth, C., 2008. Neuronal Targeting in Diabetes Mellitus: A
667 Story of Sensory Neurons and Motor Neurons. Neurosci. 14, 311–318.
668 https://doi.org/10.1177/1073858408316175

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678 **Captions to illustrations**

679 **Figure 1.** Illustration of the experimental protocol to record the HD-sEMG signals.

680 **Figure 2.** Illustrative force and EMG-related signals from a type II diabetes patient
681 without DPN (Absent group). In (a), the 64-channel matrix used for recordings, from
682 which 32 channels (8x4 matrix, represented in the figure by the black rectangle) were
683 used to record the electromyographic signals from the tibialis anterior muscle. In (b), the
684 recordings consisted of force and 28 differentials signals acquired during isometric ankle
685 dorsiflexion at 30% MVIC. In (c), action potentials propagation, discharge rate, and spike
686 train of three motor units obtained after HD-sEMG signal decomposition.
687

688 **Figure 3.** (a) Motor unit conduction velocities (MUCV) defined from a sequence of
689 surface EMG channels from single participants of the Control, Absent, Moderate, and
690 Severe groups. (b) Discharge rates of decomposed motor units (MU) from single
691 participants of the Control, Absent, Moderate and Severe groups.
692

693 **Figure 4.** Boxplot of motor unit conduction velocity (a) and motor unit territory area (b),
694 for the different groups investigated. In all boxplots, center lines represent the median
695 value, and the box limits illustrate the lower and upper quartiles (the 25th and 75th
696 percentiles). The upper and lower whiskers extend to 1.5x the interquartile range, and
697 data beyond the end of the whiskers are outliers, shown as dots.
698

699 **Figure 5.** Boxplot of motor unit discharge rates (a) and coefficient of variation of force
700 (b), for the different groups investigated. In all boxplots, center lines represent the median
701 value, and the box limits illustrate the lower and upper quartiles (the 25th and 75th
702 percentiles). The upper and lower whiskers extend to 1.5x the interquartile range, and
703 data beyond the end of the whiskers are outliers, shown as dots.
704

705 **Table 1.** Demographic and clinical data of the participants. Different letters represent
706 statistically significant differences at $p < 0.05$.
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